

QUALITY ASSURANCE/QUALITY CONTROL
SM 9020- 2005 (As published in SM 22nd Edition)

Facility Name: _____ VELAP ID _____

Assessor Name: _____ Inspection Date _____

Records Examined: SOP Number/ Revision/ Date _____ Analyst: _____

Sample ID: _____ Date of Sample Preparation: _____ Date of Analysis: _____

Relevant Aspect of Standards	Method Reference	Y	N	N A	Comments
Thermometers					
1) Is the necessary calibration correction factor marked on each temperature measuring device so that only calibrated-corrected temperature values are recorded?	9020B.4.a.				
2) Is the accuracy of the reference certified thermometer verified as specified on the certificate of calibration or at least every 5 years?	9020B.4.a.				
Balance					
3) Does the balance provide a sensitivity of at least 0.1 g at a load of 150 g, with weights traceable to appropriate national standards? Is an analytical balance used having a sensitivity of 1 mg under a load of 10 g for weighing small quantities (less than 2 g) of materials?	9030B.7				
4) Is a balance zero check done with each use?	9020B.4.b				
5) Are working weights checked annually against a set of reference weights of known tolerance (e.g., ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision, and linearity?	Table 9020:1				
6) Are reference weights recertified as specified in the certificate of calibration or at least every 5 years?	9020B.4.b				
7) Is balance serviced annually or more often as conditions change or problems occur?	9020B.4.b				
pH Meter					
8) Is a digital meter used, graduated in ≤ 0.1 pH units, that includes temperature compensation? Are commercial buffer solutions and electrodes suitable for a wide temperature range used?	9020B.4.c & 9030B.6				
9) Is the pH meter calibrated before each use with at least two certified pH buffers that bracket the pH of sample being measured? Is the pH of the test solution measured at a temperature close to the temperature used to calibrate the meter?	9020B.4.c				

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
10) Are buffer solutions or single-use/ready-to-use pH solution packets discarded immediately after being used to calibrate meter? Are all buffer solutions made from packets discarded after 1 day?	9020B.4.c				
11) Is pH electrode stored immersed in solution recommended by manufacturer?	9020B.4.c				
12) Is pH meter slope measured and recorded after calibration at least once a month? If the pH meter does not have a feature that automatically calculates the slope, but can provide the pH in millivolts (mV), is the formula in 9020B.4.c used to calculate the slope?	9020B.4.c				
Mechanical media dispensing apparatus					
13) Is volume check by dispensing into a graduated cylinder at the start of each volume change?	9020B.4.f				
Autoclave					
14) Is glassware autoclaved at 121°C for at least 30 min? Are all bottle caps loosened before autoclaving?	9040				
Hot-air sterilizing oven (If needed, see referenced sections)	9020B.4.g and 9040)				
Refrigerator					
15) Is temperature maintained at 2 to 8°C with thermometer bulb in distilled water or glycerol solution?	9020B.4.i				
Freezer (If needed, see referenced sections)	9020B.4.j)				
Membrane Filtration Equipment					
16) Are filter funnel and membrane holders made of seamless stainless steel, glass, or autoclavable plastic, and leak-proof and are not scratched or corroded? Are broken metal grids replaced?	9030B.16				
17) Are reusable membrane filter units checked for accuracy of graduation marks initially using a Class A graduated cylinder or volumetric pipet?	9020B.4.k				
Water Bath Incubator					
18) Is a water-bath incubator with a gabled cover to reduce water and heat loss and a circulating pump to maintain set temperature used? Are weights or screens used on plastic bags containing plated media to permit total immersion?	9030B.1				
19) Is a total immersion thermometer graduated in 0.1°C used for 44.5±.02°C incubation temperatures?	9020B.4.a & n.				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
20) Is water bath filled only with reagent-quality water? Is water level maintained so that it is above the upper level of the medium in tubes or flasks?	9020B.4.n				
Incubator (air, water-jacketed, or aluminum block)					
21) When culture incubation is conducted in an incubating room, is the room equipped with heating units, forced air circulation, and air exchange ports? Is temperature recorded in areas where plates or tubes are incubated? Are open metal wire or perforated shelves spaced to ensure temperature uniformity throughout the chamber? Is there a 2.5-cm space between walls and stacks of dishes or baskets of tubes?	9030B.1.				
22) Is calibration corrected temperature checked and recorded on the shelves in use, or at least one on the top shelf and one on the bottom shelf, to ensure temperature consistency throughout unit? If a glass thermometer is used, is the bulb and stem submerged in water or glycerin to the immersion mark?	9020B.4.o				
Conductivity meter					
23) Is meter calibrated monthly using a certified low-level standard at 25°C or by determining cell constant using a certified low-level standard at 25°C? When solutions must be measured at a different temperature, is a meter used with automatic temperature compensation or is the temperature of solution taken, reading recorded, and then reading corrected to 25°C using the formulae in Section 2510B.5b (usually 2%/°C)?	9020B.4.q				
Optical Counting Equipment					
24) When counting colonies, is either a mechanical hand tally or an electronic tally unit that touches the agar surface used? Is the electronic tally used after any colony culture transfer, if needed, has occurred? Is electronic tally tested before initial use and annually thereafter for accuracy?	9030B.5.c				
Inoculating Equipment					
25) Are ≥3 mm diameter wire loops made of 22 or 24 gauge nickel alloy or platinum-iridium for flame sterilization used?	9030B.18				
26) Are prepackaged sterile disposable plastic loops or single-service hardwood or plastic applicators, 0.2 to 0.3 cm in diameter and at least 2.5 cm longer than the fermentation tube used?	9030B.18				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
27) Are wooden applicators sterilized by dry heat and plastic applicators by autoclave, while stored in glass or other nontoxic containers?	9030B.18				
Dilution Water Bottles					
28) Are bottles or tubes closed with glass stoppers or screw caps equipped with non toxic liners?	9030B.13				
29) Are graduation levels marked indelibly on side of dilution bottles or tubes?	9030B.13				
30) Are plastic bottles of acceptable size substituted for glass provided that they can be sterilized properly?	9030B.13				
Dilution Water Blanks					
31) Is each batch or lot, checked at least one per batch or lot, or a set percentage, e.g., 1 to 4%, for pH (7.2 ± 0.1) and volume (99 ± 2 mL)? Are dilution water bottles examined for a precipitate and discarded if present?	9020B.5.c				
Petri Dishes					
32) For HPC are 100 X 15 mm or 150 X 20 mm glass or plastic petri dishes used? Do dishes have bottoms that are free from bubbles and scratches and are flat so the medium will be of uniform thickness throughout the plate?	9030B 14				
33) For the membrane filter technique, are 60 X 15 mm loose-lid glass or plastic dishes, or 50 X 12 mm tight-lid dishes used?	9030B.14				
34) Are glass petri dishes sterilized and stored in metal cans (aluminum or stainless steel, but not copper), or wrapped in sulfate pulp (kraft) paper before sterilizing?	9030B.14				
Sample Bottles					
35) If preparing sample bottles in-house, use reusable bottles of glass or plastic made of nontoxic materials, such as polypropylene.	9030B.19				
36) Is one sample bottle per lot checked for auto-fluorescence properties (if used for fluorescence testing)?	9020B.5.d				
Pipets, Micropipets, and Graduated Cylinders					
37) Is the error of calibration for a given manufacturer's lot no more than 2.5%?	9030B.9				
38) Is a pipet aid used and there is no pipetting by mouth?	9030B.9				
Multi-well trays and sealers					

Notes/Comments:

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39) Is sterility of multi-well trays checked for at least one per lot by aseptically adding 100 mL of non-selective medium, sealed, and incubated at 35±0.5°C for up to 48 h?	9020B.5.e				
40) Is sealing performance of heat sealer unit evaluated monthly by adding one to two drops of a food-color dye to 100 mL deionized water sample, running through sealer, and visually checking each well for leakage?	9020B.5.e				
Washing And Sterilization					
41) Is all contaminated laboratory ware sterilized, and then all markings removed before cleaning?	9040				
42) Is stainless steel or other nontoxic material used for the rinse-water system? Is copper plumbing not used to distribute water?	9040				
Reagent-grade water					
43) Does reagent water quality for the microbiology laboratory meet the limits in Table 9020:II? <u>Monthly Tests:</u> Conductivity <2 µmhos/cm (µSiemens/cm) at 25°C Total organic carbon <1.0 mg/L Total chlorine residual use <0.1 mg/L Heterotrophic plate count (SM 9215B) <500 CFU/mL <u>Annual Tests</u> Single metals (Cd, Cr, Cu, Ni, Pb, and Zn) <0.05 mg/L Total metals <0.10 mg/L	9020B.5.f				
44) OPTIONAL: The Use Test for evaluation of new sources of reagent water and new lots media and membranes as described in 9020B.5.f.	9020B.5.f				
Reagents					
45) Are Material Safety Data Sheets (MSDS) provided with reagents, standards and media maintained and available to all personnel?	9020B.5.g & j				
Membrane filters and pads					
46) Are membrane lots rejected and manufacturer informed if the following criteria are not met? After sample incubation, colonies should be well-developed with appropriate color and shape as defined by the test procedure. The gridline ink should not channel growth along the ink line nor restrict colony development. Colonies should be distributed evenly across the membrane surface.	9020B.5.i				
Culture media					

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments														
47) Are media ordered in quantities to last no longer than 1 yr?	9020B.5.j																		
48) Is date of expiration and date opened placed on containers?	9020B.5.j																		
49) Are dehydrated media stored in a tightly closed container in a cool (15 to 25°C), dry, controlled temperature room or desiccator away from direct sunlight?	9020B.5.j																		
50) Are media prepared in clean containers that are at least twice the volume of the medium being prepared?	9020B.5.j.1)																		
51) After rehydrating a medium, are media dispensed promptly to culture vessels and sterilized within 2 h?	9050A.3.																		
52) Are media sterilized at 121°C maximum for minimum time specified? <table><tr><td><u>Material</u></td><td><u>Time in min at 121°C</u></td></tr><tr><td>Membrane filters and pads</td><td>10</td></tr><tr><td>Carbohydrate-containing media (e.g. lauryl tryptose, BGB broth)</td><td>12–15</td></tr><tr><td>Contaminated materials and discarded cultures</td><td>30</td></tr><tr><td>Wrapped membrane filter assemblies and empty sample collection bottles</td><td>15</td></tr><tr><td>Buffered dilution water, 99 mL in screw-cap bottle</td><td>15</td></tr><tr><td>Rinse water, volume >100 mL</td><td>Adjust time for volume</td></tr></table>	<u>Material</u>	<u>Time in min at 121°C</u>	Membrane filters and pads	10	Carbohydrate-containing media (e.g. lauryl tryptose, BGB broth)	12–15	Contaminated materials and discarded cultures	30	Wrapped membrane filter assemblies and empty sample collection bottles	15	Buffered dilution water, 99 mL in screw-cap bottle	15	Rinse water, volume >100 mL	Adjust time for volume	9020B.5.j.2) & Table 9020:IV				
<u>Material</u>	<u>Time in min at 121°C</u>																		
Membrane filters and pads	10																		
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Wrapped membrane filter assemblies and empty sample collection bottles	15																		
Buffered dilution water, 99 mL in screw-cap bottle	15																		
Rinse water, volume >100 mL	Adjust time for volume																		
53) Are media containing carbohydrates not exposed to the elevated temperatures for more than 45 min? Exposure time is defined as the period from initial exposure to heat to removal from the autoclave. Are autoclave printout records maintained?	9020B.5.j.2)																		
54) Are media not reautoclaved?	9020B.5.j.2)																		
55) Are melted agars tempered before use in a water bath at <50°C, preferably 44 to 46°C, for ≤3 h? Is agar temperature monitored by exposing a bottle of water or medium containing a thermometer to the same heating and cooling conditions as the agar?	9020B.5.j.3)																		
56) If agar medium is not used immediately but allowed to solidify for storage, is agar media remelted in boiling water, flowing steam, or low-wattage microwave? Is any unused melted agar discarded? Is agar melted only once?	9020B.5.j.3)																		
57) Are agar plates inverted as soon as poured agar has solidified?	9020B.5.j.3)																		

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
58) Are agar plates stored in sealed plastic bags or other sealed container if they will be held more than 2 d?	9020B.5.j.4)				
59) Are media used within holding times below? <u>Medium</u> Broth in tightly closed screw-cap flasks at 2-8°C 96 h Poured agar in plates with tight-fitting covers at 2-8°C 2 weeks Agar or broth in loose-cap tubes at 2-8°C 2 weeks Poured agar plates with loose-fitting covers inverted in sealed plastic bags at 2-8°C 2 weeks Agar or broth in tightly closed screw-cap tubes at < 30°C 3 months Large volume of agar in tightly closed screw-cap flask or bottle at 2-8°C 3 months	9020B.5.j.4), Table 9020:V & 9020B.5.j.4)				
60) Are prepared media containing dyes protected from light and excessive evaporation? If color changes occur, are media discarded?	9020B.5.j.4) & 9050A.1				
61) Is the liquid level marked in several tubes (10% of each batch) after sterilization and monitored for lost weight or volume if stored for more than 2 weeks? If loss is 10% or more, is batch discarded?	9020B.5.j.4)				
62) Are all petri dishes with solid media discarded if they are dried out, e.g., wrinkled, cracked, or pitted?	9020B.5.j.4)				
63) If media are refrigerated, are they brought to room temperature before use and the batch rejected if growth or false positive responses are present?	9020B.5.j.4)				
64) Is a complete record of each batch of laboratory prepared medium maintained with date and name of preparer, name and lot number of medium, amount of medium weighed, volume of medium prepared , sterilization time and temperature, pH adjustments needed, final pH, and preparations of labile components?	9020B.5.j.6)				
Dilution Water					
65) Is stock phosphate buffer solution prepared as follows? Dissolve 34.0 g KH ₂ PO ₄ in 500 mL reagent-grade water, adjust to pH 7.2 ± 0.5 with 1N NaOH, and dilute to 1 L with reagent-grade water. Is stock solution sterilized by filtration or autoclave, store refrigerated and discarded if turbidity develops?	9050C.1.a.1)				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
66) Is MgCl_2 stock solution prepared as follows? Add 38 g/L MgCl_2 OR 81.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ to 1 L reagent grade water. Is stock solution sterilized, stored in refrigerator and discarded if solution becomes turbid?	9050C.1.a.2)				
67) Is dilution water working solution prepared as follows? Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride stock solution to 1 L reagent-grade water. Dispense in amounts that will provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min. Final pH should be 7.2 ± 0.1 . Note that pH values will change with time. Is dilution water stored refrigerated after opening, discarded if turbidity develops and used within 6 months?	9050C.1.a.3)				
68) Is peptone water, 0.1%, prepared as follows? Prepare by adding 1 g peptone to 1 L reagent water. Final pH should be 7.0 ± 0.2 after sterilization. Dispense in amounts to provide 99 ± 2.0 mL or 9 ± 0.2 mL after autoclaving for 15 min. Is peptone water stored refrigerated after opening, discarded if turbidity develops and used within 6 months?	9050C.1.b.				
69) Is each new batch (or lot, if commercially prepared) of buffered water checked for sterility before first use by adding 50 mL of the water to 50 mL of a double-strength tryptic soy, trypticase soy, or tryptose broth. Alternatively, is sterility checked by aseptically passing 100 mL or more of dilution water through a membrane filter and placing filter on nonselective medium? Is filter incubated at $35 \pm 0.5^\circ\text{C}$ for 24 h and observed for growth? NOTE: Dilution water and membrane filter rinse water are the same and require the same QC check.	9020B.9.d, 9222B.2.c.				
70) Is sample suspended in dilution water for ≤ 30 min at room temperature?	9050C.1.				
Analytical Quality Control Procedures for Established Methods					
71) Are certified reference cultures in Table 9020:VI used for testing media?	9020B.9.b.				
72) Are duplicate analyses of plate colonies performed at least monthly or more frequently as needed, e.g., 10% of samples when required by the analytical method or regulations, one sample per test run, or one sample per week for a laboratory that conducts less than 10 tests/week? NOTE: A test run is defined as an uninterrupted series of analyses.	9020B.9.c				
73) Is precision of replicate analyses calculated for each different type of sample examined (drinking water, ambient water, or wastewater) according to the procedure outlined at SM 9020B.9.e?	9020B.9.e.				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
Verification	9020B.10				
Multiple-tube fermentation (MTF) confirmation methods	9020B.10.a.				
<u>74) Drinking Water</u> —Carry tests through confirmed phase only. The Completed Test is not required.	9020B.10.a.1) a)				
<u>75) Drinking Water</u> - For QC purposes, if normally there are no positive results within a quarter, is at least one positive source water sample analyzed to confirm that the media and laboratory procedures and equipment produce appropriate responses?	9020B.10.a.1) a)				
<u>76) Drinking Water</u> - For samples with a history of heavy growth without gas in presumptive-phase tubes, are the tubes carried through the confirmed phase to check for false negative responses for coliform bacteria? Are any positives verified for thermotolerant (fecal) coliforms or <i>E. coli</i> ?	9020B.10.a.1) a)				
<u>77) Other water types</u> —Is verification achieved by performing the completed phase at a frequency established by the laboratory, such as 10% of positive samples, or one sample per test run, or a certain percentage depending on normal laboratory work load?	9020B.10.a.1) b)				
<u>78) Fecal streptococci procedure</u> - Is verification performed as outlined in Section 9230C.5 at a frequency established by the laboratory?	9020B.10.a.2)				
Membrane Filter Verification Methods	9020B.10.b				
<u>79) Total coliform procedures in drinking water</u> : Is entire membrane swabbed or five typical and five atypical (nonsheen) colonies picked up from positive samples on M-Endo or LES-Endo agar medium and verified as in Section 9222B.4f?	9020B.10.b.1) a),				
<u>80) Drinking Water</u> : Are any positive total coliform colonies verified for thermotolerant (fecal) coliforms as specified in SM9222B.4.f. OR verified for <i>E. coli</i> in accordance with 40 CFR 141.21(f)(6)(i) or (ii) or one of the alternative methods listed in appendix A to subpart C of 40 CFR 141?	9020B.10.b.1) a), 40 CFR 141.21(f)(6)				
<u>81) If there are no positive samples, is at least one known positive source water sample tested quarterly?</u>	9020B.10.b.1) a)				
<u>82) Total coliform procedures in water other than drinking water</u> : Are positives verified monthly by picking at least 10 typical and atypical colonies from a positive water sample as in Section 9222B.4f? Are counts adjusted based on percent verification?	9020B.10.b.1) b)				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
83) Are false negatives determined in any water type by picking representative atypical colonies of different morphological types and verifying as in Section 9222B.4f?	9020B.10.b.1) c)				
84) Thermotolerant (fecal) coliform procedure (SM 9222D) – Are positives verified monthly by picking at least 10 blue colonies from one positive sample using lauryl tryptose broth and EC broth as in Section 9221E.1b? Are counts adjusted based on percent verification?	9020B.10.b.2)				
85) Are false negatives determined by picking representative atypical colonies of different morphological types and verifying as in Presumptive Phase, Section 9221B.2?	9020B.10.b.2)				
86) <i>E. coli</i> procedure for water other than drinking water - Is one positive sample verified monthly by picking from well-isolated colonies while taking care not to pick up medium, which can cause a false positive response? Are citrate test and the indole test performed as described in Section 9225D.4 and 7, or other equivalent identification procedures or systems? Is indole test incubated at 44.5°C? <i>E. coli</i> are indole positive and yield no growth on citrate. Are counts adjusted according to percentage of verification?	9020B.10.b.3) b)				
87) Are false negatives determined by picking representative atypical colonies of different morphological types and verifying as in SM 9020B.10.b.3)b)?	9020B.10.b.3) c)				
88) <u>Fecal streptococci procedure</u> : At least monthly, are at least 10 isolated red colonies from m-Enterococcus agar picked to brain heart infusion (BHI) media and tested as described in Section 9230C? Are counts adjusted according to percentage of verification?	9020B.10.b.4)				
89) <u>Enterococci procedures</u> - Are at least 10 well-isolated pink to red colonies with black or reddish-brown precipitate from EIA agar picked to verify monthly and transfer to BHI media as described in 9230C? Are counts adjusted according to percentage of verification?	9020B.10.b.5)				
Enzyme defined substrate tests	9020B.10.c				
90) Enterococci—Are colonies verified by selecting 10 typical colonies (positives) and 10 atypical colonies (negatives) once per month or 1 typical and 1 atypical colony from 10% of positive samples, whichever is greater?	9020B.10.c.3)				
Samples	9060				

Notes/Comments:

Relevant Aspect of Standards	Method Reference	Y	N	NA	Comments
91) Are samples rejected if sample bottles do not have at least 2.5 cm of air space in the bottle and resampling requested? Alternatively, are overfilled samples added to a larger sterile sample bottle in the laboratory to assure adequate mixing?	9060A.3				
92) For drinking water samples, is a minimum of 100 ±2.5 mL collected?	9060A.4.				
93) <i>Drinking water for compliance purposes:</i> For total coliform and <i>E. coli</i> analyses, is the holding time from collection to analysis no more than 30 h?	9060B.1.b				
94) Are samples for heterotrophic plate count analysis maintained at <8°C and do not exceed 8 h holding time?	9060B.1.b				
95) For bacterial samples in wastewater sludge (fecal coliforms and <i>Salmonella</i> sp.), is the regulatory holding time 24 h?	9060B.1.c				

Notes/Comments: